20. A method of manufacturing a therapeutic composition, said method comprising the steps of bringing together an immunotoxin according to claim 16 and a pharmaceutically acceptable adjuvant or carrier.--

REMARKS

With entry of this amendment, claims 12-20 are under examination. The claims have been rewritten to overcome 35 USC § 112, first and second paragraph rejections, and not to distinguish from any prior art. No new matter has been added. Reconsideration is requested.

New claim 12 is directed to the new and inventive peptide fragment consisting of the first 1020 amino acids of the *Clostridium sordellii* lethal toxin (LT). Although the complete amino acid sequence of LT was known, the claimed peptide fragment is novel over the prior art. Moreover, the peptide fragment is nonobvious, since it has surprising and unexpected properties in that the said peptide fragment modifies and inactivates the GTP-binding protein Ras which is a signal protein in cancer cells playing a pivotal role in cancer cell proliferation. Both modification and inactivation of Ras by the inventive peptide fragment is nonobvious over the pertinent prior art.

New claim 13 is directed to a combination of the peptide fragment according to claim 1 and a target cell specific binding domain. The subject matter of claim 13 is originally disclosed in the specification, on page 4, fourth paragraph. The term "immunotoxin" means, to the skilled person, that a target cell specific binding domain is present together with a "toxic principle" which, in the present invention, is the peptide fragment according to claim 12.

New claim 14 is a combination of the peptide fragment according to claim 12, a translocation domain, and a target cell specific domain.

New claim 15 is directed to a specific translocation domain as originally disclosed in the specification on page 10, at the end of paragraph four.

New claim 16 is directed to the subject matter of former dependent claim 14.

New claims 17-20 are directed to the same subject matter as former claims 7 and 10.

The drawings were objected to as being informal. Applicants request that the requirement for formal drawings be deferred until allowable subject matter has been indicated.

The Examiner indicated that the requirements for sequence listings had not been satisfied. Filed herewith is a substitute Sequence Listing in paper and computer-readable form. Although the Examiner indicated that a "Notice to Comply" was included with the Office Action, it was not received by Applicants' representative, to whom the official papers were mailed by the PTO.

An abstract is submitted herewith to satisfy the requirements for an abstract.

The Examiner indicated that the title of the application was not descriptive. Clarification is requested. Applicants submit that the title describes the invention disclosed and claimed. However, the Examiner's suggestions for an alternative title are welcome.

The disclosure was objected to for certain informalities. With regard to the Examiner's indication that the priority claim was not recited in the first line of the specification, the Examiner's attention is drawn to item 14 of the filing papers.

Certified copies of the European and PCT priority documents are being obtained and will be submitted to the Examiner in due course.

The Brief Description of the Drawings has been amended and is believed to be complete. The spelling corrections have been made on page 1, paragraph 2.

Claims 7-10 were rejected under 35 USC § 112, second paragraph, as being indefinite. The claims have been cancelled and new claims 12-20, submitted herewith, are believed to be free of the rejection. Favorable reconsideration is requested.

Claims 7-10 were rejected under 35 USC § 112, first paragraph, as being overly broad. This rejection is rendered moot by cancellation of these claims. New claims 12-20 are clearly drawn, and Applicants believe that these claims are not overly broad.

Claims 7-8 and 10 were rejected under 35 USC § 102(b) as being anticipated by Popoff or Roberts et al., as evidenced by Chaves-Olarte et al.

Popoff (1987) describes a method of preparing the lethal Toxin (LT) of *Clostridium sordellii* from culture supernatants of the growing backterium. The paper does not contain any anticipation or even suggestion as to any catalytic activity of the toxin. Popoff (1987) does not present any DNA sequence. Moreover, Popoff (1987) does not anticipate or disclose where the catalytic domain of the toxin might be located.

As stated on page 3 §3 ff, Popoff (1987) presents a protocol for purification of LT and phenomenology of LT action. However, a protocol of preparing an enzymatically active fragment of the holotoxin (as is known for the diphtheria toxin a-chain) was not disclosed, nor was the sequence of LT known at that time, nor was its mode of action known.

Roberts describes bacteria or bacterial components used for vaccination against several *Clostridial* species including *C.sordellii*. Since the application under discussion is directed towards the use of the catalytic activity of the lethal toxin (LT) of *C.sordellii* (i.e. the functional blockade of the GTP-binding protein Ras especially

in its ontogenic version) as a pharmacologically active principle, the disclosure of Roberts does not suggest combination with Popoff (1987).

Chaves-Olarte (JBC 1999) was published in April 1999, and two of the inventors herein are authors of the paper. As the subject U.S. application was filed in the United States on July 31, 1998, with a priority date of February 2, 1996, and the paper by Chaves-Olarte was received for publication on November 30, 1998, it is clear that the paper by Chaves-Olarte was published after the filing date of the subject application. Applicants respectfully request that Chaves-Olarte be withdrawn as a reference.

Applicants submit that claims 12-20 are not anticipated by Popoff (1987) or Roberts as evidenced by Chaves-Olarte, for the reasons set forth above.

Claims 7-8 and 10 were also rejected as being anticipated by Green et al. or von Eichel-Streiber et al as evidenced by Chaves-Olarte et al.

The paper by Green et al., in Gene (1995) 161: 57-61, teaches the DNA-sequence of the lethal toxin of *C. sordellii* and presents evidence for a homology to *C. difficile* toxins A and B. The paper does not present any evidence or experiments related to the mode of action of LT, which is the main focus of patent application WO 97/27871. Nor is the glucosylation of Ras mentioned or even predicted.

As stated on page 3 of the subject patent application, Green et al published the DNA and derived amino acid sequence of LT of *C.sordellii* strain 6018. There is no disclosure of the mode of action nor the position of the catalytic domain of LT.

Moreover, from the paper of Green et al., one cannot deduce that the N-terminal 1020 amino acids carry the transferase enzyme activity, as claimed herein. Applicants respectfully submit that claims 12-20 are clearly distinguished from the teaching of Green et al.

Von Eichel-Streiber et al teach that for the cytotoxin of *C.difficile* the N-terminal 1020 AS should bear the catalytic domain. It is anticipated that a similar fragment of LT carries the catalytic component of this toxin. No evidence whatsoever is given on the substrate and cosubstrate specificities of LT, nor was the mode of any of the LCTs known at the time of publication.

This paper relates to sequencing of the variant *C.difficile* toxin TedB-1470 which exerts an overall effect on cells that is similar to that of the LT of *C.sordellii*. As can be deduced from Chaves-Olarte (see above JBC 1999), the variant toxin Tod-1470 and the lethal toxin TcsL-1522 share some of their GTPase substrates. However, the protooncogene Ras (to specify h-Ras) is not shared.

As mentioned on page 3 of the subject application, von Eichel-Streiber et al (Mol.Micro, 1995) have presented data on a *C.difficile* toxin ToxB-1470 that induces morphological effects identical to LT, but both clearly different from those of *C.difficile* ToxB. However, again these authors did not disclose the mode of action nor the substrates of LT.

As mentioned on page 2-3 of the subject specification, Giry et al (Infect.Immun. 1995) have presented data indicating that the Rho, Rac, Cdc42 were the substrates of toxins A and B of C. difficile but not of LT of *C.sordellii*.

These publications show that neither the activity of LT as a glucosyl-transferase nor its substrates are anticipated thereby.

Applicants respectfully submit that claims 12-20 submitted herewith are allowable.

Claims 8-9 were rejected under 35 USC § 103 as being unpatentable over Popoff (1987) or von Eichel-Streiber in view of Blakely et al. Claims 8-9 were also rejected under 35 U.S.C. § 103 as being unpatentable over Green et al. in combination

with Vitetta et al. or Sandhu. The primary references, Popoff (1987), von Eichel-Streiber and Green have been discussed and distinguished above. Applicants respectfully submit that these rejections are moot in view of the submission of new claims 12-20.

All objections and rejections having been addressed, it is respectfully submitted that the application is in condition for allowance, and Notice to that effect is respectfully requested.

Respectfully submitted,

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